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Transformation of group A streptococci by electroporation

Alexander Suvorov [1], Jan Kok [2] and Gerard Venema [2]

[1] *Institute of Experimental Medicine, Leningrad, U.S.S.R., and* [2] *Institute of Genetics, University of Groningen, Haren, The Netherlands*

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1. SUMMARY

The introduction, via electroporation, of free plasmid DNA into three strains of *Streptococcus pyogenes* is described. The method is very simple and rapid and efficiencies vary from 1×10^3 to 4×10^4 per μg of DNA. The method was also used to introduce an integrative plasmid and transformants were obtained, albeit at a somewhat lower frequency (2×10^2). Some of the plasmids used in this study are derivatives of the *Lactococcus lactis* subsp. *cremoris* Wg2 plasmid pWV01. These broad host range vectors replicate in Gram-positives as well as Gram-negatives (viz. *Escherichia coli*). Here we show that they also replicate in *S. pyogenes* and *S. sanguis*.

2. INTRODUCTION

Group A streptococci (*Streptococcus pyogenes*) are important human pathogens causing a variety of serious inflammatory diseases, sometimes followed by acute rheumatic fever or poststreptococcal glomerulonephritis. Their importance in view of medical care has resulted in an intensification of research aimed at the elucidation of the genetic background of these organisms. Only recently, this has led to the identification, cloning and sequencing of a number of genes, amongst which several M protein genes [1–5], the genes for streptolysin [6] and streptococcal exotoxin A [7,8], the Immunoglobulin G-receptor gene [9], and the hyaluronidase gene from an *S. pyogenes* bacteriophage [10]. A major drawback in the genetic research in this field is the fact that no direct system for transformation of group A streptococci is available. Transduction and conjugation have been shown to operate in these organisms (for a review see Wannamaker [11], and [12]). Using conjugative mobilization, Scott et al. [13] were able to transfer the *emm*6 gene, coding for type 6 M protein, to an M^- strain of *S. pyogenes*, which became functionally M^+.

The development of a transformation system for group A streptococci is essential, however, for further rapid increase in the knowledge of the factors involved in virulence and pathogenicity. This would offer the possibility of applying recombinant DNA technologies to the group A streptococci, without using an intermediate host, and would allow studies of protein and gene structure and function in the natural environment. In this communication we demonstrate the uptake of free plasmid DNA by two strains of *S. pyogenes* using the electroporation technique, a method suc-

Correspondence to: Jan Kok, Institute of Genetics, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands.

cessfully applied to an increasing number of microorganisms [14–20]. In addition, we show that vectors based on the cryptic *Lactococcus lactis* subsp. *cremoris* plasmid pWV01 are able to replicate in group A and H (*S. sanguis*) streptococci. Because these vectors also replicate in *Escherichia coli* and *Bacillus subtilis* [21], these observations open a new avenue for future genetic studies in group A streptococci.

3. MATERIALS AND METHODS

3.1. Strains, plasmids and media

The strains and plasmids used in this study are summarized in Table 1. All strains were routinely grown at 37°C in Brain Heart Infusion (BHI) broth with 5% horse serum (both from Difco, U.S.A.). Plasmids were isolated on a miniprep scale essentially as described by Birnboim and Doly [22]. To digest the cell wall of overnight cultures, the cells were incubated for 20 min at 37°C in lysis solution containing 10 mg/ml lysozyme and 30 U/ml mutanolysin. Agarose gel electrophoresis and restriction enzyme digestions were done as described by Maniatis et al. [23].

Table 1

Strains and plasmids used in this work

Strain	Phenotype	Source
Streptococcus pyogenes (Lancefield group A)		
154	Tet^R, Rif^R	Lab collection, Leningrad
1800	M18	Lab collection, Leningrad
CS112	M^-, Str^R	P. Cleary, University of Minnesota, USA
Streptococcus sanguis (Lancefield group H)		
challis 57		H. Malke, CIMET, Jena, GDR
challis GS10.1		Lab collection, Leningrad
Plasmid (size in kb)	**Marker [a]**	**Reference**
pGK12 (4.5)	Em^R, Cm^R	[21]
pGK13 (4.9)	Em^R, Cm^R	pGK12 derivative [21] Lab collection, Groningen
pGK20 (5.9)	Em^R, Cm^R	pGK13 with *lacZ* gene fusion, Lab collection, Groningen
pVI101 (7.4)	Em^R	Golubkov et al. [28]
pGT128 (5.4)	Em^R	Golubkov et al. [24]
pPPCV5 (4.3)	Em^R	Lab collection, Leningrad

[a] Em^R: erythromycin resistance; Cm^R: chloramphenicol resistance.

3.2. Transformation

Transformation of *Streptococcus sanguis* was done according to Golubkov et al. [24] except that *S. sanguis* was grown in BHI without sheep erythrocytes.

3.3. Electroporation

Electroporation was done essentially as described by van der Lelie et al. [19]. An overnight culture of *Streptococcus pyogenes* in Todd Hewitt Broth (Difco, U.S.A.) supplemented with 40 mM dl-threonin was diluted 100-fold in the same medium and grown to an optical density at 650 nm of 0.2 ($5–8 \times 10^8$ cells per ml). For electroporation, the cells in 25 ml of the culture were washed twice in electroporation buffer (EB: 5 mM phosphate buffer, pH 6.9, 0.3 M sucrose, 1 mM $MgCl_2$), resuspended in 2 ml EB and cooled on ice for at least 15 min. DNA was added to 0.6 ml of the cell suspension and electroporation was carried out at 2500 V, 25 μF in a Gene Pulser™ (Bio-Rad Laboratories). After the electric pulse, the cells were kept on ice for 10 min and subsequently diluted 10-fold in BHI, containing 0.3 M sucrose and 5% horse serum. After allowing time for expression (2 h at 37°C), the cells were plated onto BHI-agar plates containing 5 μg of erythromycin per ml. Transformant colonies were visible after 48 h of incubation at 37°C.

4. RESULTS

4.1. Electroporation

To investigate whether electroporation was possible in group A streptococci, *Streptococcus pyogenes* strain 154 was chosen as the model strain. Various plasmids were used as donor molecules. These are listed in Table 1 and all carry an erythromycin resistance gene as the selectable marker. After the first pilot experiments, in which we found some Em^R colonies with plasmid pGK13, we decided to use this plasmid, a broad host range

vector derived from a cryptic plasmid of *Lactococcus lactis* subsp. *cremoris* [21], to optimize the system parameters. We found that the best electroporation results for strain 154 were obtained when logarithmically growing cells were used (OD at 650 nm approximately 0.2; $5–8 \times 10^8$ cells per ml). A 100-fold diluted overnight culture of the strain usually reached this optical density after 2.5 to 3 h of growth at 37°C. The rate of survival of the cells after the high-voltage electric pulse was 40–55% in the various experiments. The number of transformants varied from 10^4 to 10^5 per μg of DNA (Table 2). With the streptococcal vectors pVI101 and pGT128 transformation frequencies of 10^3 and 5×10^3 were obtained, respectively (Table 2). Using the same conditions of electroporation, we were able also to introduce plasmid DNA in the *S. pyogenes* strains CS112 and 1800 (Table 2). Even after several trials, no transformants of strain 1800 with plasmid pGK13 were found. In all cases, the plasmid content of several transformants was checked using a plasmid minipreparation procedure. The molecular mass of the plasmids in the transformants was the same as the original plasmids used for the electroporation, as deduced from restriction enzyme analysis and subsequent agarose gel electrophoresis (Fig. 1). Furthermore, restriction enzyme analysis confirmed that no major rearrangements had occurred during or after the uptake process (results not shown). Plasmid pGK13 contains, apart from the erythromycin resistance gene, a gene conferring resistance to chloramphenicol in all the hosts it has been transferred to. Both resistance genes originate from *Staphylococcus aureus*. To investigate whether the Cm^R gene also functions in *S. pyogenes*, a culture of *S. pyogenes* strain 54(pGK13) was diluted and plated onto BHI-agar plates with increasing amounts of chloramphenicol. The Cm^R gene was expressed in this strain with a MIC value of approximately 10 μg/ml.

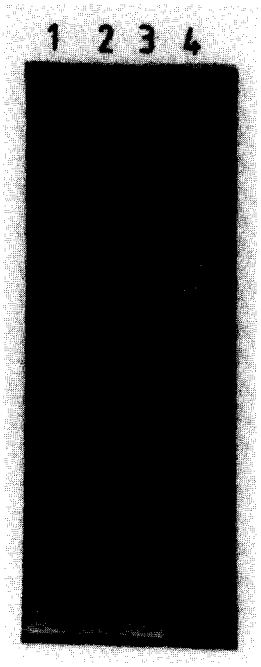

Fig. 1. Agarose (0.8%) gel electrophoresis of *Eco*RI digests of pVI101, (lane 1); pGT128, (lane 2); pGK13, (lane 3). Lane 4, size reference: phage lambda DNA digested with *Hin*dIII.

Table 2

Efficiencies of transformation via electroporation of group A streptococci

Plasmid (size in kb)	Strain of *S. pyogenes*		
	154	1800	CS112
pGK13 (4.9)	4×10^4	0	2×10^3
pVI101 (7.4)	5×10^3	6.5×10^3	NT
pGT128 (5.4)	1×10^3	3×10^3	NT
pPCVI1 (4.3)	NT	NT	2×10^2

NT: not tested; efficiencies are expressed as number of transformants per μg of DNA.

The integrative plasmid pPPCV5 was used in the electroporation of *S. pyogenes* strain CS112. Erythromycin resistant colonies were found with a frequency of 2×10^2 per μg DNA. No plasmid DNA was found after miniprep DNA analysis, suggesting that the plasmid had integrated into the chromosome of the recipient cells.

4.2. Transformation of S. sanguis *with* Lactococcus *derived vectors*

The cryptic plasmid pWV01 from *Lactococcus lactis* subsp. *cremoris* Wg2 has been used to construct a series of small versatile antibiotic resistance vectors [21]. The plasmids are extremely useful as they bridge the gap between Gram-posi-

tives and Gram-negatives and replicate in members of both groups (e.g. *Escherichia coli, Bacillus subtilis* and lactic acid bacteria). As shown above, derivatives of pWV01 also replicate in group A streptococci. To see whether it was possible to introduce these plasmids in the naturally transformable *Streptococcus sanguis,* we chose the challis strains 57 and GS10.1 as the recipients in transformation experiments. pWV01 derivatives pGK12, pGK13 and pGK20 were used as well as a control plasmid: pVI101. All plasmids efficiently transformed both strains to erythromycin resistance. The number of transformants per μg of DNA ranged from 1 to 2.6×10^4 for the pWV01 derivatives and was 3×10^5 for pVI101. Plasmid pGK20 is a pWV01 derivative carrying an in-frame fusion between the 9th codon of the *L. lactis* subsp. *cremoris* Wg2 proteinase gene [25] and the *E. coli lacZ* gene. Transcription and translation of the fused genes is directed by signals derived from the proteinase gene (J. Kok, unpublished). When plated on BHI-agar plates supplemented with 0.004% 5-bromo-4-chloro-3-indolyl-β-galactoside (X-gal), all the Em^R resistant transformants from pGK20 show up as blue colonies, indicative or *lacZ* gene expression and β-galactosidase activity in the *S. sanguis* transformants.

5. DISCUSSION

The lack of a method to introduce free DNA into group A streptococci is a major drawback in the dissection of the genetics of the diseases these pathogens are involved in. Until now, only one report deals with an indirect method of DNA transfer [13]. A cointegrate shuttle vector, a derivative of the *E. coli*/streptococcus plasmid pVA838, was used to transform *S. sanguis* challis. After introduction of pVA797, a conjugative plasmid which has homology with pVA838, this challis host was used as a donor in matings with an *S. pyogenes* recipient. It is clear that an efficient direct method of transformation of group A streptococci would be of eminent importance. The electroporation protocol described here is the first example of such a procedure and it offers the possibility to apply recombinant DNA technology to these organisms. Although the efficiency of transformation is only moderate, an increase can be expected soon from the optimization of the physical parameters of the electroporation equipment.

The fact that pWV01 derived vectors replicate in *S. pyogenes* offers a number of possibilities which might accelerate genetic studies in these organisms. A large number of small cloning vectors have been constructed from pWV01, some of which contain multiple cloning sites, some allowing cloning with marker inactivation (of antibiotic resistance genes). With some derivatives LacZ α-complementation is possible in *E. coli.* Some of the vectors can be used to screen for promoter and terminator sequences and a number of expression vectors have been developed (van de Guchte, pers. comm. [21,26,27]). The most important property of the vectors, however, is the fact that they replicate in Gram-positives (e.g. *Bacillus subtilis*) as well as in *E. coli.* For these two bacterial species, advanced genetic engineering techniques have been developed and, through the pWV01 vector system, these are directly linked to the nascent field of genetics of group A streptococci.

The introduction of the integrative plasmid pPPCV5 is the first step in a better understanding of the genetic organization of the chromosome of *S. pyogenes,* because it offers the possibility to isolate mutants, and, subsequently, to clone the gene(s) involved in the mutation. Furthermore, the strategy could be used to stably insert (in vitro manipulated) genes in the chromosome. In this way, the functioning of factors involved in the starting and development of pathogenicity, i.e. the role of nucleases, proteases, hyaluronidase, neuramidase, streptolysin and streptokinase can be studied in the natural environment. Ultimately, the development of efficient recombinant DNA techniques for group A streptococci could lead to the construction of artificial vaccine strains, carrying a set of antigens raising an immune response to most types of streptococci.

Only a few possibilities are mentioned here, and we are confident that the method to introduce and express free DNA in *S. pyogenes* which we describe here will be of great importance to future fundamental and applied research.

REFERENCES

[1] Scott, J.R. and Fischett, V.A. (1983) Science 221, 758–760.

[2] Hollingshead, S.K., Fischetti, V.A. and Scott, J.R. (1986) J. Biol. Chem. 261, 1677–1686,.

[3] Spanier, J. and Cleary, P. (1985) Science 225, 935–938.

[4] Kehoe, M.A., Poirier, T.P., Beachey, E.H. and Timmis, K.N. (1985) Infect. Immun. 48, 198–203.

[5] Haanes-Fritz, E., Robbins, J.C. and Cleary, P. (1987) in Streptococcal Genetics (Ferretti, J.J. and Curtiss III, R., eds.), pp. 106–108, Am. Soc. Microbiol., Washington, D.C.

[6] Kehoe, M.A. and Timmis, K.N. (1984) Infect. Immun. 43, 804–810.

[7] Weeks, C.R. and Ferretti, J.J. (1986) Infect. Immun. 52, 144–150.

[8] Johnson, L.P., L'Italien, J.J. and Schlievert, P.M. (1986) Mol. Gen. Genet. 203, 354–356.

[9] Heath, D.G. and Cleary, P.P. (1987) in Streptococcal Genetics (Ferretti, J.J. and Curtiss III, R., eds.), pp. 121–123, Am. Soc. Microbiol., Washington, D.C.

[10] Hynes, W.L. and Ferretti, J.J. (1987) in Streptococcal Genetics (Ferretti, J.J. and Curtiss III, R., eds.), pp. 150–152, Am. Soc. Microbiol., Washington, D.C.

[11] Wannamaker, L.W. (1982) in Microbiology (Schlessinger, D., ed.), pp. 112–116, Am. Soc. Microbiol., Washington, D.C.

[12] Scott, J.R., Guenthner, P.C., Malone, L.M. and Fischetti, V.A. (1986) J. Exp. Med. 164, 1641–1651.

[13] Scott, J.R., Hollingshead, S.K., Jones, K.F. and Fischetti, V.A. (1987) in Streptococcal Genetics (Ferretti, J.J. and Curtiss III, R., eds.), pp. 93–97, Am. Soc. Microbiol., Washington, D.C.

[14] Shivarova, N., Förster, W., Jacob, H.-E. and Grigorova, R. (1983) Z. Allg. Mikrobiol. 23, 595–599.

[15] Harlander, S. (1987) in Streptococcal Genetics (Ferretti, J.J. and Curtis III, R., eds.), pp. 229–233, Am. Soc. Microbiol., Washington, D.C.

[16] MacNeil, D.J. (1987) FEMS Microbiol. Lett. 42, 239–244.

[17] Chassy, B.M. and Flickinger, J.L. (1987) FEMS Microbiol. Lett. 44, 173–177.

[18] Powell, I.B., Achen, M.G., Hillier, A.J. and Davidson, B.E. (1988) Appl. Environ. Microbiol. 54, 655–660.

[19] Van der Lelie, D., van der Vossen, J.M.B.M. and Venema, G. (1988) Appl. Environ. Microbiol. 54, 865–871.

[20] Fiedler, S. and Wirth, R. (1988) Anal. Biochem. 170, 38–44.

[21] Kok, J., van der Vossen, J.M.B.M. and Venema, G. (1984) Appl. Environ. Microbiol. 48, 726–731.

[22] Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523.

[23] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

[24] Golubkov, V.I., Gupalova, T.V., Kolesnichenko, T.G., Iontova, I.M., Malke, H. and Totolian, A.A. (1982) in Microbiology (Schlessinger, D., ed.), pp. 180–182, Am. Soc. Microbiol. Washington, D.C.

[25] Kok, J., Leenhouts, K.J., Haandrikman, A.J., Ledeboer, A.M. and Venema, G. (1988) Appl. Environ. Microbiol. 54, 231–238.

[26] Van der Vossen, J.M.B.M., Kok, J. and Venema, G. (1985) Appl. Environ. Microbiol. 50, 540–542.

[27] Vosman, B., Kooistra, J., Olijve, J. and Venema, G. (1987) Gene 52, 175–183.

[28] Golubkov, V.I., Reichardt, W., Boitsov, A.S., Iontova, I.M., Malke, H. and Totolian, A.A. (1982) Molec. Gen. Genet. 187, 310–316.